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(54) Polypeptide for use in treatment of autoimmune disease

(57) The use of a polypeptide comprising an amino acid sequence not homologous to a sequence synthesised by the cells of the patient, for the manufacture of a medicament for the treatment of an autoimmune disease is described.

1 Autoimmune Disease Treatment

2

3 This invention relates to the treatment of autoimmune
4 diseases, and especially the prophylactic treatment of
5 such diseases.

6

7 Stress of a varied nature, induced as a result of heat
8 shock, nutrient deprivation, oxygen radicals and other
9 forms of metabolic disruption, including infection by
10 certain viruses, bacteria and protozoans, as well as
11 certain cases of cellular transformation, all lead to
12 the increased synthesis of a family of proteins
13 collectively known as stress proteins or heat shock
14 proteins.

15

16 These stress proteins are among the most highly
17 conserved and abundant proteins found in nature.
18 Further these proteins have been shown to be among the
19 dominant antigens recognised in immune responses to a
20 broad spectrum of pathogens. A review of the
21 interrelationships between stress proteins, infection
22 and immune surveillance has recently appeared, in
23 which a clear analysis of these relationships is
24 provided (13).

25

1 It has become apparent in recent years that a
2 relationship exists between so-called stress or heat
3 shock proteins and certain immune responses to
4 infection and to the development of autoimmunity. As
5 an example, the analysis of cell-mediated and humoral
6 responses to a variety of bacterial and parasitic
7 pathogens has shown that heat shock proteins are often
8 strongly immunogenic during infection (1-8).
9

10 Proteins involved in immune responses to certain
11 parasitic diseases such as malaria, schistosomiasis,
12 leishmaniasis, trypanosomiasis and filariasis, have
13 been identified as members of the hsp 70 and 90 gene
14 families. Further antigens related to hsp 70 and GroEL
15 families have been shown to play a role in T cell and
16 B cell recognition during bacterial infections
17 including leprosy, tuberculosis and Q. fever. The
18 mycobacterial GroEL stress protein has been identified
19 as the target of a T cell clone capable of causing
20 autoimmune disease in a rat model of adjuvant-induced
21 arthritis (9). Similar results have been obtained as
22 concerns the small heat shock proteins, since an
23 immunologically important 19 Kd protein antigen of
24 Mycobacterium leprae has been sequenced, and shown to
25 have considerable amino acid sequence homology to the
26 soybean 19Kd heat shock protein.
27

28 Elevated responses to the GroEL stress protein have
29 been found by testing T cells from synovial infiltrates
30 of rheumatoid arthritis patients (10). Autoantibodies
31 to hsp 90 have been reported in systemic lupus
32 erythematosus (SLE) (11). In addition, elevated
33 antibody responses to hsp70 and GroEL stress proteins
34 have been found in SLE and in rheumatoid arthritis
35 (12).

1
2 The stress proteins are remarkable in their
3 evolutionary conservation: hsp90, hsp70, and hsp60
4 proteins are found in all prokaryotes and eukaryotes.
5 In fact comparison of almost any two hsp70 proteins
6 from two different organisms indicates an amino acid
7 homology of around 50%. The major stress proteins
8 occur at low levels in normal, unstressed cells, but
9 accumulate to very high levels in cells undergoing
10 stress. A striking example is the case of *E. coli*
11 hsp60, which accounts for 1.6% of total cell protein
12 under normal growth conditions, and can accumulate to
13 15% of total cell protein after heat shock (14).
14 Stress proteins appear to fulfil vital roles in cells,
15 both in the absence and in the presence of stress.
16 They appear to be involved in the assembly and
17 disassembly of protein complexes, and hsp70 proteins
18 are important for the translocation of certain
19 proteins through cellular membranes (15). Stress
20 proteins appear to interact with many different
21 proteins, for example, hsp90 has been found to interact
22 with steroid hormone receptors and with viral and
23 cellular kinases. Hsp70 proteins bind to DNA
24 replication complexes, clathrin baskets, the cellular
25 tumour antigen p53, and immunoglobulin heavy chains.
26 Plant hsp60 interacts with Rubisco, which fixes CO₂ in
27 chloroplasts, and may be the most abundant protein in
28 the biosphere (16). The interaction of stress
29 proteins with multiple proteins may provide an
30 explication for the evolutionary constraints imposed
31 on their amino acid sequences.
32
33 Stress proteins have an almost certain role in
34 protecting cells and organisms from the deleterious
35 effects of heat and other stresses.

1
2 It seems clear that the tight sequence regulation
3 imposed on many heat shock protein sequences throughout
4 evolution has led to such retained sequences between
5 those of the host and those of the infectious agent
6 having a significant degree of identity. Clearly the
7 reaction of the host immune system against antigens of
8 the infecting organism could lead to the raising of
9 antibodies against heat shock proteins. The sequence
10 homology within the heat shock protein family thus
11 points to conserved sub-sequences of heat shock
12 proteins as being serious candidates for inducing an
13 immune response that can have specificity against self
14 sequences, with the consequence of inducing an
15 autoimmune reaction and the associated disease states.
16

17 The reports referenced above indicate that stress
18 proteins, such as the heat shock proteins, provide
19 particularly attractive targets for immune recognition.
20 An analysis the cross reactivity of T cell responses to
21 stress proteins has been published recently (17),
22 wherein the presence of human T cells was demonstrated
23 that were capable of immune recognition of conserved
24 sequence determinants. These authors have proposed a
25 model in which immune responses to stress proteins
26 provide a link between infectious and autoimmune
27 diseases.
28

29 Although models of the role of stress proteins in
30 autoimmune diseases have been proposed, no-one has yet
31 suggested possible treatment for autoimmune diseases.
32

33 In accordance with a first aspect of the present
34 invention a method of treating an autoimmune disease in
35 a patient comprises introducing a compound, comprising

1 an amino acid sequence of a protein which is not
2 homologous with amino acid sequences synthesised by
3 cells of the patient, into the patient.

4

5 In accordance with another aspect of the present
6 invention there is provided use of a compound
7 comprising an amino acid sequence of a protein for the
8 treatment of an autoimmune disease in a patient,
9 wherein the amino acid sequence is not homologous with
10 amino acid sequences synthesised by cells of the
11 patient.

12

13 Further, the invention provides a composition for
14 treatment of an autoimmune disease in a patient,
15 comprising a compound which comprises an amino acid
16 sequence of a protein which is not homologous with
17 amino acid sequences synthesised by cells of the
18 patient, in combination with a pharmaceutical carrier.

19

20 Still further, the invention provides the use of a
21 compound comprising an amino acid sequence of a protein
22 which is not homologous with amino acid sequences
23 synthesised by the cells of a patient for the
24 manufacture of a medicament for the treatment of an
25 autoimmune disease in the patient.

26

27 Preferably, the compound comprises a peptide which
28 comprises the amino acid sequence and typically, the
29 protein is a stress or heat shock protein.

30

31 Preferably, the treatment is prophylactic.

32

33 Typically, the compound could be introduced into a
34 patient by incorporation in a cream or ointment, in a
35 soluble glass, in slow release capsules, transdermal

1 patches, injected, or even administered orally or in
2 suppository form.

3

4 Preferably, the amino acid sequence has antigenic
5 properties.

6

7 The amino acid sequence could be naturally occurring or
8 be synthesised. If the amino acid sequence is
9 synthesised then the peptide could comprise a number of
10 different amino acid sequences and/or multiples of the
11 same amino acid sequence.

12

13 The invention described here is based on the
14 above-detailed conservation of heat shock sequences and
15 their implication in autoimmune diseases. Contrary to
16 the identity of certain conserved sequences, this
17 invention, is based on the hypervariable sequences of
18 stress proteins. Prior immunisation with natural or
19 synthetic peptides representing such non-conserved,
20 variable or hypervariable stress protein sequences of
21 origin from infectious agents of bacterial and other
22 parasitic pathogens, induces antibody responses
23 against the stress proteins of the infecting organism,
24 and these specifically induced antibodies are incapable
25 of recognising self stress protein sequences. The
26 rapid recognition of infectious agent - specific stress
27 proteins by specific pre-existing antibodies raised
28 against non-homologous peptides from invading stress
29 proteins should allow the elimination of these stress
30 proteins before they are able to elicit potentially
31 autoimmune responses.

32

33 This invention concerns the immune recognition of
34 peptide epitopes of specific heat shock or stress
35 proteins, and the development of peptide-based therapy

1 or prevention based on such epitopes.

2

3 Examples of the invention will now be described.

4

5 1. Analysis of stress protein peptide sequences

6

7 In order to practice the preventive/therapeutic

8 approach described in this invention, it is necessary

9 to examine in detail the amino acid sequences of human

10 heat shock proteins, and of those of organisms

11 infecting human beings with whom correlations of immune

12 diseases exist.

13

14 Our initial approach was to assemble a table of certain

15 of the known sequences of stress proteins from human

16 and infectious agent sources. A selection of these

17 sequences are presented in Appendix 1. A thorough

18 analysis of sequence homology between members of each

19 of the stress protein families indicates that for

20 each of the principle stress protein families, hsp70,

21 hsp90 and hsp27, certain sequences have been highly

22 conserved throughout evolution, whereas parts of the

23 stress proteins contain amino acid sequences that are

24 highly differentiated. One assumes that the

25 conservational pressures concerning the retained

26 sequences are associated with critical structural or

27 functional aspects of these important proteins. The

28 variable regions are presumably of less critical

29 structural or functional importance, thus escaping

30 from the conservative pressure/selection activities

31 prevailing in evolving organisms.

32

33

34 2. Selection of candidate peptide vaccines

35

1 The selection of useful candidate peptides capable of
2 eliciting an immune response specifically against the
3 stress proteins of the infectious agent is based on
4 two major criteria:

5

6 i) The non-identity of selected peptide sequences,
7 and their lack of resemblance to highly, or partially
8 conserved stress protein sequences, common to human
9 and infectious agent proteins. The selection of such
10 non-conserved sequences is derived from a reverse
11 analysis of amino acid sequence homologies, in other
12 words, concentrating on the non-homologous sequences
13 evident from homology analyses such as those shown in
14 (1) and in appendix 2.

15

16 For a thorough selection of sequence differences
17 versus sequence homology, it is instructive to, in
18 addition to amino acid identity, to look at
19 replacements by highly conserved amino acids. Examples
20 of such substitutions are the following groups:
21 (aspartic acid and glutamic acid), (lysine and
22 arginine), (serine and threonine), (phenylalanine and
23 tyrosine), and (isoleucine, leucine, valine and
24 methionine).

25

26 ii) An analysis of the antigenic potential of selected
27 peptide sequences. Where information is available,
28 peptide epitopes that conform to the criteria of both
29 points i) and ii), and which can be demonstrated to be
30 immunodominant, are preferred examples of the
31 preventive/therapeutic peptides described in this
32 invention.

33

34 Examples of the amino acid sequences of some selected
35 peptides that reply to the criteria of point i) are

1 presented in appendix 2.

2
3 Examples of group i) peptides that are expected to
4 have considerable immunogenic potential have been
5 selected on the basis of presently accepted criteria
6 of immunological potential. Examples of certain
7 peptides with pronounced antigenicity are shown in
8 appendix 3.

9
10 Non-homologous sequence comparison of the known stress
11 protein and related antigen sequences from humans and
12 from infectious agents has been performed. In the case
13 of Plasmodium falciparum, in addition to regions of
14 extensive homology of amino acid sequence between the
15 two proteins, clear regions of extensive lack of
16 homology are also detectable, and the following
17 sequence fragments, depicted using the one and
18 three-letter amino acid abbreviations derived from the
19 IUPAC-IUB Commission on Biochemical Nomenclature (see
20 Table 1), illustrate this example:-

21
22 ALIGNMENT OF RESIDUES 133 TO 254 OF 75KDa antigen of P
23 Falciparum TO RESIDUES 357 TO 635 OF HSP70 HUMAN

24
25 ENYCYGVKSSLEDKIKEKLQP~~A~~EIETCMKTIT~~T~~ILEWLEKNQLAGKDEYE
26 ----- KNALES-Y-AFNMKSA- VEDEG LKGKIS-E

27
28 AKQKEAESVCAPIMSKIY-QDAA-GAAGGMPGGM-P-GGMPGGMP GGMNF
29 ADKKKVLDKCQEVIS- WLDANTLA EKDEFEHKRKELEQVCNPIISGL-Y

30
31 PG-GMPG-AGMPGNAP---AGSGPTVEEVV
32 QGAGGPGPGGFGAQGPKGGSGSGPT-----

33
34 Examples of non-homologous peptides are shown in bold
35 letters. The second peptide of HSP70 human shown in

1 bold above, denoted "Peptide example 1", has been
2 compared to the sequence of the corresponding antigen
3 of Mycobacterium tuberculosis and its highly unique
4 sequence has little or no counterpart in the sequence
5 of tubercular origin.
6

7 ALIGNMENT OF RESIDUES 8 TO 11 OF PEPTIDE 1 TO RESIDUES
8 1 TO 127 OF 71kDa antigen M. tuberculosis
9

10 K----R--K-- E-----
11 KEDIDRMIKDAEAHAEEDRKREEADVRNGAETL VYNTEKFVKEQREGG
12

13 Clearly other peptide sequences unique to an infectious
14 agent antigen exist and will have value in the
15 applications described in this invention. In order to
16 identify such sequences, extensive cloning, expression
17 and sequence analysis of infectious agent antigens
18 will be required. Such research, although technically
19 arduous, is quite within the realms of existing
20 technology. Similarly, once new sequences are
21 established, the presence or absence of amino acid
22 sequence homologies can be determined either
23 visually, or through the use of any number of amateur
24 or commercial sequence analysis software programs. Our
25 intention here is to demonstrate the general procedure
26 for identifying, and applying both specific
27 non-homologous and specific homologous stress and
28 infectious agent antigen peptide sequences to the
29 vaccination, therapeutic and cosmetic applications
30 described herein.
31
32
33

34 3 The Rational Design of Synthetic Peptides
35

1 This invention is not limited to naturally occurring
2 variant sequences within stress proteins, nor is it
3 limited to the selection and use of a single variant
4 epitope. For example, synthetic peptides could be
5 used. In addition, the peptide could be synthesised to
6 have combinations of different variant sequences or
7 multiples of variant sequences. By synthesising
8 peptides comprising different variant sequences and/or
9 multiples of the same variant sequence it may be
10 possible to design peptides having a stronger immune
11 response against stress proteins of infectious
12 organisms but which do not recognise human stress
13 epitopes.

14 A recent analysis of variant peptide epitopes of
15 myelin basic protein (MBP), and their influence on the
16 incidence of experimental autoimmune encephalomyelitis
17 (EAE) has indicated that synthetic variants of an
18 N-terminal MBP peptide can have greatly altered
19 properties of binding to cell surface glycoproteins
20 encoded by the major histocompatibility complex (MHC)
21 (18). In other words, the efficacy of the complex
22 interactions associated with the elicitation of an
23 effective immune response against peptide antigens, can
24 be altered and improved in some cases, by the use of
25 synthetic variants of natural antigens. The subject
26 of this invention comprises those variant peptide
27 sequence approaches that are taught by the authors
28 of reference 18, amongst others.

29
30 An efficient mapping procedure for identifying protein
31 antigenic determinants has been described that would
32 be of use in the selection of useful antigenic
33 determinants for the applications taught in this
34 invention (19). Clearly classical chemical, enzymatic
35

1 and combined synthetic procedures can be utilised to
2 produce candidate peptides, once identified and
3 selected, for the vaccine applications described here.
4 A naturally expected limitation of the peptide vaccines
5 that can be produced using this described procedure
6 derives from the fact that about one third of
7 monoclonal and polyclonal antibodies made by
8 immunising with native protein react with assembled
9 topographic sites (20). These assembled determinants
10 may not form the appropriate structure outside of a
11 proteins native environment. This limitation is not
12 expected to significantly limit the practical use of
13 this invention.

14

15 Studies concerning T Cell recognition and activation
16 have indicated that it may be possible to design
17 peptides with predictable and advantageous properties
18 (21). These authors have described two approaches for
19 immunomodulation that could be useful for the design
20 of therapeutic strategies against autoimmune
21 encephalomyelitis. The first approach consists of a
22 thorough molecular characterisation of an
23 encephalitogenic epitope, and the subsequent design of
24 peptide analogs that retain normal or increased major
25 histocompatibility complex binding properties, and that
26 fail to activate disease-inducing T cells. Secondly,
27 novel properties of a heterocyclic peptide have been
28 described, with the result that the peptide is highly
29 antigenic in vitro, while being non-immunogenic in
30 vivo. These authors have been able to demonstrate the
31 feasibility of immune intervention in an immune disease
32 through the use of a synthetic peptide. These results
33 are complementary to the procedure we describe here,
34 but are not identical, nor do they in any way predict
35 the approach that we describe.

1
2 4 Applications of the stress protein peptides described
3 herein
4
5 The basic tenant that we have developed herein is based
6 on the multiple observations that certain infectious
7 agent antigens are closely related in amino acid
8 sequence to human stress proteins, and that immune
9 reactions against such antigens can cross react with
10 the human proteins, leading to the possibility of
11 developing autoimmune disease. Our invention describes
12 the selection of stress protein peptide sequences from
13 infectious agent antigens related to human stress
14 proteins, but which have little or no sequence homology
15 within such human stress proteins. The injection of
16 such non-homologous peptides into human beings, for
17 instance in an emulsification with Freunds complete
18 adjuvant, would provide a route of effective
19 vaccination against subsequent autoimmune disease
20 induced as mentioned above. The antibodies raised
21 through such vaccination are specific to the selected
22 infectious agent antigen from which the vaccinating
23 peptide was derived. Such induced antibodies are
24 specific to infectious agent antigens, thus explaining
25 their efficacy in the application of this invention.
26
27 Further, since the vaccinating agent is a small
28 peptide, instead of a large, complex protein such as
29 human factor VIII, or factor IX, it is not compulsory
30 to use an injection as a means of delivering the
31 peptide to a human subject. We thus reserve in our
32 application the administration of the kinds of peptides
33 described by transdermal applications, a number of
34 which are presently commercialised with considerable
35 success.

1
2 Further still, since certain major diseases that are
3 thought to have their origin in autoimmune diseases,
4 such as arthritis and rheumatism, the peptides of this
5 invention can be applied externally, in both local and
6 cosmetic application to painful joints and
7 articulations resulting from these prevalent diseases.
8

9 For example, the peptides could be administered to a
10 patient by incorporation in a cream or ointment, in a
11 soluable glass, in slow release capsules, transdermal
12 patches, injected, or even administered orally or in
13 suppository form.

14
15 In addition, due to the nature of amino acid sequences
16 it is unlikely that treatment using these substances
17 will produce the unpleasant side effects which are
18 normally associates with drugs.

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1 APPENDIX 1

2
3 NON-HOMOLOGOUS SEQUENCES WHICH ARE ALSO
4 KNOWN ANTIGENICS ARE DENOTED BY
5 UNDERLINING AND NON-HOMOLOGOUS ONLY
6 SEQUENCES ARE DENOTED BY BOLD LETTERING
7
8

9 SEQUENCE OF HUMAN STRESS PROTEINS
1011
12 A) Sequence HSP90 Human
13

14 Rebbe N F, Ware J, Bertina M, Modrich P, Stafford D W
15 Gene 53:235-245(1987)
16 EMBL; M16660; HSHSP90
17 KW Heat Shock. Sequence 724 AA; 83293 MW
18
19
20

| | | | | | |
|----|-------------|------------|-------------|------------|-----|
| 21 | MPPEEVHHGEE | EVETFAFQAE | IAQLMSLIIN | TFYSNKEIFL | 40 |
| 22 | RELISNASDA | LDKIRYESLT | DPSKLDMSGKE | LKIDIIPNPQ | 80 |
| 23 | ERTLTLVDTG | IGMTKADLIN | NLGTIAKSGT | KAFMEALQAG | 120 |
| 24 | ADISMIGQFG | VGFYSAYLVA | EKVVVIRKHN | DDEQYAWESS | 160 |
| 25 | AGGSFTVRAD | HGEPIGMGTK | VILHLKEDQT | EYLEERRVKE | 200 |
| 26 | VVKKHSQFIG | YPITLYLEKE | REKEISDDEA | EEEKGEKEEE | 240 |
| 27 | DKDDEEKPKI | EDVGSDEEDD | SGKDKKKKTK | KIKEKYIDQE | 280 |
| 28 | ELNKTKPWT | RNPDDITQEE | YGEFYKSLTN | DWEDHLAVKH | 320 |
| 29 | FSVEGQLEFR | ALLFIPRRAP | FDLFENKKKK | NNIKLYVRRV | 360 |
| 30 | FIMDSCDELI | PEYLNFIRGV | VDSEDLPLNI | SREMLQQSKI | 400 |
| 31 | LKVIRKNIVK | KCLELFSELA | EDKENYKKFY | EAFSKNLKLG | 440 |
| 32 | IHEDSTNRRR | LSELLRYHTS | QSGDEMTSLS | EYVSRMKETQ | 480 |
| 33 | KSIYYITGES | KEQVANSAFV | ERVRKRGFEV | VYMTEPIDEY | 520 |
| 34 | CVQQLKEFDG | KSLVSVTKEG | LELPEDEEEK | KKMEESKAKF | 560 |
| 35 | ENLCKLMKEI | LDKKVEKVTI | SNRLVSSPCC | IVTSTYGWTA | 600 |

| | | | | | |
|---|------------|------------|------------|------------|-----|
| 1 | NMERIMKAQA | LRDNSTMGYM | MAKKHLEINP | DHPIVETLRQ | 640 |
| 2 | KAEADKNDKA | VKDLVVLLFE | TALLSSGFSL | EDPQTHSNRI | 680 |
| 3 | TYMIKLGLGI | DEDEVAEEEP | NAAVPDEIPP | LEGDEDASRM | 720 |
| 4 | EEVD | | | | 724 |

5

6

7

8 B) Sequence HSP70 Human

9

10 [1] Hunt C, Morimoto R I;
 11 Proc Natl Acad Sci USA 82:6455-6459(1985)
 12 EMBL; M11236; HSHSP701
 13 EMBL; MII717; HSHSP70D
 14 KW Heat Shock
 15 Sequence 640AA; 69867 MW

16

| | | | | | |
|----|------------|-------------------|------------|-------------------|---------|
| 17 | MAKAAAVGID | LGTTYSVGV | FQHGKVEIIA | NDQGNRTTPS | 40 |
| 18 | YVAFTDTERL | IGDAAKNQVA | LNPQNTVFDA | KRLIGRKFGD | 80 |
| 19 | PVVQSDMKHW | PFQVINDGDK | PKVQVSYKGE | TKAFYPEEIS | 120 |
| 20 | SMVLTKMKEI | AEAYLGYPVT | NAVITVPAYF | NDSQRQATKD | 160 |
| 21 | AGVIAGLNVL | RIINEPTAAA | IAYGLDRTGK | GERNVLIFDL | 200 |
| 22 | GGGTFDVSIL | TIDDGIFEVK | ATAGDTHLGG | <u>EDFDNRLVNH</u> | 240 (3) |
| 23 | FVEEFKRKHK | KDISQNKRRAV | RRLRTACERF | EGIDFYTSIT | 280 |
| 24 | RARFEELAKR | <u>TLSSSTOASL</u> | EIDSLCSDLF | RSTLEPVEKA | 320 (4) |
| 25 | LRDAKLDKAQ | IHDLVLVGGS | TRIPKVQKLL | QDFFNGRDLN | 360 |
| 26 | KSINPDEAVG | YGAAVQAAIL | MGDKSENVQD | LLLLDVAPLS | 400 |
| 27 | LGLETAGGVM | TALIKRNSTI | PTKQTQIFTT | YSDNQPGVLI | 440 |
| 28 | QVYEGERAMT | KDNNLLGRFE | LSGIPPAPGV | PQIEVTFDID | 480 (1) |
| 29 | ANGILNVTAT | DKSTGKANKI | TITNDKGRLS | KEEIERMVQE | 520 |
| 30 | AEKYKAEDEV | <u>QRERVSAKNA</u> | LESYAFNMKS | AVEDEGLKGK | 560 (2) |
| 31 | ISEADKKKVL | DKCQEVISWL | DANTLAEKDE | FEHKRKELEQ | 600 |
| 32 | VCNPIISGLY | QGAGGPGPGG | FGAQGPKGGS | GSGPTIEEVD | 640 |

33

34

35

I C) Sequence Human HSP27

2

3 Hickey E, Brandon S E, Potter R, Stein G, Stein J,
4 Weber L A;

5 Nucl. Acids Res 14:4127-4145(1986)

6 EMBL;X03900; HSHSP27

7 KW: HEAT SHOCK

8 SEQUENCE 199 AA; 22327 MW;

9

| | | | | | |
|----|------------|------------|------------|------------|-----|
| 10 | MTERRVPFSL | LRGPSWDPFR | DWYPHSRLFD | QAFGLPRLPE | 40 |
| 11 | EWSQWLGGSS | WPGYVRPLPP | AAIESPAVAA | PAYSRALSRQ | 80 |
| 12 | LSSGVSEIRH | TADRWRVSLD | VNHFAPDELT | VTKTDGVVEI | 120 |
| 13 | TGKHEERQDE | HGYISRCFTR | KYTLPPGVDP | TQVSSSLSPE | 160 |
| 14 | GTLTVEAPMP | KLATQSNEIT | IPVTFESRAQ | LGGRSCKIR | 200 |

15

16 D) Sequence Human HSP60

17

18 Sequence not yet available, submitted for publication:
19 Gupta R S, Jinal S, Harley C B and Dudani A K(1989)

20

21

22 SEQUENCE OF HSP60 YEAST

23

24

25 Reading D S, Hallberg R L and Myers A M (1989). Nature
26 337 655

27

| | | | | | |
|----|-------------|------------|------------|------------|-----|
| 28 | MLRSSVVRSR | ATLRPLLRR | YSSHKILKFG | VIGRASLLKG | 40 |
| 29 | VETLAIAVAA | TLGPKGRNVL | IEQPFGPPKI | TKDGVTVAKS | 80 |
| 30 | IVLKDKFINM | GAKLLQIVAS | KTNIAAGDGT | TSATVLGRAI | 120 |
| 31 | FTISVKNVAA | GCNPMDLRRG | SQVAVIKVIL | FLSANKKEIT | 160 |
| 32 | TSEEIAQVAT | ISANGDSHVG | KLLASAMEKV | GKEGVITIRE | 200 |
| 33 | GRITTLEDELE | VTEGMRFDRG | FISPYFITDP | KSSKVEFEKP | 240 |
| 34 | LLLLSEKKIS | SIQDILPALE | ISNQSRRPLL | IIAEDVDGEA | 280 |
| 35 | LAACILNKL | GQVKVCAVKA | PGFGDNRKNT | IGDIAVLTGG | 320 |

| | | | | | |
|---|------------|------------|------------|------------|-----|
| 1 | TVFTEELDLK | PEQCTIENLG | SCDSITVTKE | DTVILNGSGP | 360 |
| 2 | KEAIQERIEQ | IKGSIDITTT | NSYEKEKLQE | RLAKLSGGVA | 400 |
| 3 | VIRVGGASEV | EVGEKKDRYD | DALNATRAAV | EEGILPGGGT | 440 |
| 4 | ALVKASRVLD | EVVVDNFDQK | LGVDIIRKAI | TRPAKQIIEN | 480 |
| 5 | AGEEGSVIIG | KLIDEYGDDF | AKGYDASKSE | YTDMLATGII | 520 |
| 6 | DPFKVVRSGL | VDASGVASLL | ATTEVAIVDA | PEPPAAAGAG | 560 |
| 7 | GMPGGMPG | MPGMM | | | 600 |

8

9 SEQUENCES OF BACTERIAL ANTIGENS

10

11

12 A) Mycobacterium leprae

13

14 18 KDa Antigen

15

16 Nerland A H, Mustapha A S, Sweetser D, Godal T, Young R
 17 J Bacteriol 170 5919-5921 (1988)
 18 Sequence 148 AA; 16643MW;

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| | | | | | |
|----|------------|------------|------------|------------|-----|
| 20 | MLMRTDPFRE | LDRFAEQVLG | TSARPAVMPM | DAWREGEFFV | 40 |
| 21 | VGFDLPGKA | DSLDIDIERD | VVTVRAERPG | VDPDREMLAA | 79 |
| 22 | ERPRGVFNRQ | LVLGENLDTE | RILASYQEGV | LKLSIPVAER | 119 |
| 23 | AKPRKISVDR | GNNGHQTINK | TPHEIIIDA | | |

24

25

26 65 KDa Antigen

27

28

29 Mehra V, Sweetser D and Young R A (1986) Proc Natl Acad
 30 Sci USA 83 7013

31

32 AA 589, MW 61,831

33 The Underling Amino Acids Correspond To Antigenic
 34 Peptides.

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| | | | | | |
|----|-------------------|--------------------|---------------------------|-------------------|-----|
| 1 | VPGRDGETQP | ASCGRPSRAL | HPASVSNNGC | RSPVILASF | 40 |
| 2 | IRRNFAMAK | TIAYDEEARR | GLERGLNSLA | <u>DAVKVTLGPK</u> | 80 |
| 3 | <u>GRNVVLEKKW</u> | <u>GAPTIITNDGV</u> | <u>SIAKEIELED</u> | PYEKIGAELV | 120 |
| 4 | KEVAKKTDDV | AGDGTTTATV | LAQALVKEGL | <u>RNVAAGANPL</u> | 160 |
| 5 | <u>GLKRGIEKAV</u> | DKVTETILLKD | AKEVETKEQI | AATAAISAGD | 200 |
| 6 | QSIGDLIAEA | MDKVGNEGVI | TVEEESNTFG | LQLELTEGMR | 240 |
| 7 | FDKGYISGYF | VIDAERQEAV | LEEPYILLVS | SKVSTVKDLL | 280 |
| 8 | PLLEKVIQAG | KSLLIIAEDV | EGEALSTLVV | NKIRGTFKSV | 320 |
| 9 | AVKAPFGFDR | RKAMLQDMAI | LTGAQVISEE | VGLTLENTDL | 360 |
| 10 | SLLGKARKVV | MTKDETTIVE | GAGDTDAIAG | RVAQIRTEIE | 400 |
| 11 | NSDSDYDREK | LQERLAKLAG | GVAVIKAGAA | TEVELKERKH | 440 |
| 12 | REIDAVRANAK | AAVEEGIVAG | GGVTLLQAAP | <u>ALDKLKLTD</u> | 480 |
| 13 | <u>EATGANIVKV</u> | ALEAPLKQIA | FNSGMEPGVV | AEKVRNLSVG | 520 |
| 14 | HGLNAATGEY | <u>EDLLKAGVAD</u> | <u>PVKVTRSA</u> <u>LO</u> | <u>NAASIAGLFL</u> | 560 |
| 15 | TTEAVVADKP | EKTAAPASDP | <u>TGGMGGMDF</u> | | 600 |

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17

18 70 KDa Antigen

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20

21 Not yet sequenced. Immunological cross-reactivity with
 22 the 71 KDa antigen of Mycobacterium tuberculosis (YOUNG
 23 ET AL Proc Natl Acad Sci USA 85, 4267-4270 (1988)).

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26 B) Mycobacterium tuberculosis

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28

29 65 KDa Antigen

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32 Schinnick, T S (1987). Journal of Bacteriology 169

33 1080

34 AA 562, MW 59083

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| | | | | | |
|----|------------|------------|-------------|------------|-----|
| 1 | RGCRHPVTTP | VSSPIRRNHF | AMAKTIAYDE | EARRGLERGL | 40 |
| 2 | NALADAVKVT | LGPKGRNVVL | EKKWGAPTIT | NDGVSIKEI | 80 |
| 3 | ELETPYEKIG | AELVKEVAKK | TDDVAGDGTT | TATVLAQALV | 120 |
| 4 | REGLRNVAAG | ANPLGLKRG | EKAVEAKVTET | LLKGAKEVET | 160 |
| 5 | KEQIAATAAI | SAGDQSIGDL | IAEAMDKVGN | EGVITVEESN | 200 |
| 6 | TFGLQLELTE | GMRFDKGYIS | GYFVTDPERQ | EAVLEDPYIL | 240 |
| 7 | LVSSKVSTVK | DLLPLLEKVI | GAGKPLLIIA | EDVEGEALST | 280 |
| 8 | LVVNKIRGTF | KSVAVKAPGF | GDRRKAMLQD | MAILTGGQVI | 320 |
| 9 | SEEVGLTLEN | ADLSLLGKAR | KVVVTKDETT | IVEGAGDTDA | 360 |
| 10 | IAGRVAQIRQ | EIENSDSDYD | REKLQERLAK | LAGGVAVIKA | 400 |
| 11 | GAATEVELKE | RKHRIEDAVR | NAKAAVEEGI | VAGGGVTLLK | 440 |
| 12 | AAPTLDELKL | EGDEATGANI | VKVALEAPLK | QIAFNSGLEP | 480 |
| 13 | GVVAEKVRNL | PAGHGLNAQT | GVYEDLLAAG | VADPVKVTRS | 520 |
| 14 | ALQNAASAIG | LFLTTEAVVA | DKPEKEKASV | PGGGDMGGMD | 560 |
| 15 | F | | | | 600 |

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18 71 KDa Antigen

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21 Partial sequence, contains only the homolgy domain with
22 HSP70

23

24 Young D, Lathigra R, Hendrix R, Sweetser D, Young R,
25 Proc Acad Sci
26 USA 85, 4265-4270 (1988).

27

| | | | | | | |
|----|------------|--------------------|-------------------|------------|-----|-----|
| 28 | EFQPSVQIQV | YQGEREIAAH | NKLLGSFELT | GIPPAPRGIP | 40 | (1) |
| 29 | QIEVTFDIDA | NGIVHVTAKD | KGTGKENTIR | IQEGLSKE | 80 | |
| 30 | DIDRMKDAE | <u>AHAEEEDRKRR</u> | <u>EEADVRNGAE</u> | TLVYNTEKFV | 120 | 3,4 |
| 31 | KEQREGGSKV | PEDTWRIGHT | GHQVGDGEAG | PGVAGSGASD | 160 | (2) |
| 32 | LRSSSGCVTG | HWRCPPRAAA | GRCPPLGM | | 200 | |

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1 C) *Plasmodium falciparum* (MALARIA)

2

3

4 90 KDa Antigen

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6

7 M Jendoubi, S Bonnefoy, Nucl Acids Res 16, 10928 (1988)
8 Partial sequence, contains only the region of homology
9 with HSP90

10

| | | | | | |
|----|------------|------------|-------------|------------|-----|
| 11 | KDFDGKKLKC | CTKEGLDIHH | SEEAKKDFET | VIKDVLHKKV | 40 |
| 12 | EKVVVCQRIT | DSPCVLVTSE | FGWSANMERI | MKAQALRDNS | 80 |
| 13 | MTSYMLSKKI | MEINARHPII | SALKQKADAD | KSDKTVKYLI | 120 |
| 14 | WLLFDTSLLT | SGFFALEEPT | TFSKRIHRCMI | KLGLSIDEEE | 160 |
| 15 | NNDIDLPPL | ETVDA | DSKMEEV | | 200 |

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17

18 75 KDa Antigen

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20

21 Ardeshir F, Flint J E, Richman S and Reese R T, Embo J.
22 6, 493-499
23 (1987).

24 Partial sequence from the first AA

25

| | | | | | |
|----|------------|-----------------|----------------|------------|-----|
| 26 | MLKLIERN | IPAKKSQIFT | TYADNQPGV | IQVYEGERAL | 40 |
| 27 | TKDNNLLGKF | HLDGIPFAPR | KVPQIEVTFD | IDANGILDVT | 80 |
| 28 | AVEKSTGKQN | HITITNDKGR | LSQDEIDRMV | NDAEKYLAED | 120 |
| 29 | EENRKRIEAR | NSLEN | YCYGKSSLEDKIKE | KLQPAEIETC | 160 |
| 30 | MKTIT | TILEWLEKNQLAGKD | EYEAKQKEAE | SVCAPIMSKI | 200 |
| 31 | YQDAAGAAGG | MPGGMPGGMP | GGMPGGMNFP | GGMPGAGMPG | 240 |
| 32 | NAPAGSGPTV | EEVVD | | | 280 |

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APPENDIX 2

DIFFERENTIATION OF HOMOLOGOUS (UNDERLINE) AND NON-HOMOLOGOUS SEQUENCES

A) Alignment of Residues 47 to 161 of partial sequence of P.Falciparum 90KD to residues 581 to 699 of human HSP90

10
11 --RI-DSPCVLVTSEFGWSANMERIMKAQALRDNSMTSYMLSKKIMEINAR
12 NRLVSSPCCIVTSTYGTANMERIMKAQALRDNSTMGYMMAKKHLEINPD
13

14 HPIISALKOKADADKSDKTVKYLIWLLFDTSSLTSGFFALEEPTTFSKRI
15 HPIVETLRQKAEADKNDKAVKDLVULLFETALLSSG-FSLEDPQTHSNRI
16

17 HRMIKLGLSIDEEE---NN
18 YRMIKLGLGIDEDEVAEE

21 B) Alignment of residues 7 to 157 of partial sequence
22 of P. falciparum 70 KDa to residues 411 to 613 of human
23 HSP70.

25 -----NTTIPAKKSOIFTTYADNQPGVLIQVYEGERALTKDNLLGKEHL
26 ALIKRNSTIPTKQTQIFTTYSQNQPGVLIQVYEGERAMTKDNLLGRFEI
27

28 DGIPPAPRKVPOIEVTFDIDANGILDVTAVEKSTGKQNHIITNDKGRLS
29 SGIPPAP-GVPQIEVTFDIDANGILNVTATDKSTGKANKITITNDKDRLS
30

31 QDEIDRMVNDAEKYLAEDEENRKRIEARNNSLENYCGYVKSSLEDK-IKEKLQ
32 KEEIERMVQEAEKYKAEDEVQRERVSAKNALESYAFNMKSAVEDEDEGLKGKIS

34 PAETCMK---TITTLEWLEKNQLAGKDEEAKQKEAESVCAPIMSKIYODA
35 EADKKVLDKQEVI-SWLDANTLAEKDEFEHKRKELEQVCNPIISGLYYOGA

1
2 C) Alignment of residues 5 to 110 of M. tuberculosis 71K
3 to residues 430 to 548 of human HSP70
4
5 ~~-----VQIOVYQGEREIAAHNKLLGSFELTGIPPA~~RGIP~~OIEVTFDI~~
6 ~~YSDNQPGVLIQVYEGERAMTKDN~~NLLGRFELSGIP~~PAP-GVPQIEVTFDI~~
7
8 ~~DANGIVHVTAKDKGTGKENTIRI~~QEGSG-LSKEDIDRM~~IKDAEAHAEEDR~~
9 ~~DANGILNVTATDKSTGKANKITITNDKGR~~LSKEEIERMVQEA~~KYKAED~~
10
11 KRREEADVRNGAE-----
12 VQRERVSAKNALESYAFNM
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APPENDIX 3

3 Antigenic Peptides of the 65 Kda Antigen of
4 Mycobacterium leprae
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6 MEHRA V, SWEETSER D and YOUNG R A (1986) Proc Natl Acad
7 Sci USA 83 7013
8
9 -NSLADAVKVTLGPGRNVVLEKKWGAPTTNDGVS
10 -RNVAAGANPLGLKRGIEKAV
11 -ALDKLKLTGDEATGA
12 -GEYEDLLKAGVADP
13 -ASDPTGGMGGMDF
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1 TABLE 1

2 One and Three Letter Amino Acid Abbreviations

| | | | |
|----|---|-----|--|
| 6 | A | Ala | Alanine |
| 7 | C | Cys | Cysteine |
| 8 | D | Asp | Aspartic acid |
| 9 | E | Glu | Glumatic acid |
| 10 | F | Phe | Phenylalanine |
| 11 | G | Gly | Glycine |
| 12 | H | His | Histidine |
| 13 | I | Ile | Isoleucine |
| 14 | K | Lys | Lysine |
| 15 | L | Leu | Leucine |
| 16 | M | Met | Methionine |
| 17 | N | Asn | Asparagine |
| 18 | P | Pro | Proline |
| 19 | Q | Gln | Glutamine |
| 20 | R | Arg | Arginine |
| 21 | S | Ser | Serine |
| 22 | T | The | Threonine |
| 23 | V | Val | Valine |
| 24 | W | Trp | Tryptophane |
| 25 | Y | Tyr | Tyrosine |
| 26 | B | Asx | Asp or Asn (not distinguished) |
| 27 | Z | Glx | Glu or Gln (not distinguished) |
| 28 | | | |
| 29 | X | X | Undetermined or atypical amino acid |

33 From: IUPAC-IUB Commission on Biochemical
 34 Nomenclature, J Biol
 35 Chem 243, 3557-3559, 1968.

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2 gene homologous to a major heat shock protein. Mol
3 Cell Biol 7 1271.

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CLAIMS

- 1
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- 4
- 5 1. A method of treating an autoimmune disease in a patient comprises introducing a compound, comprising an amino acid sequence of a protein which is not homologous with amino acid sequences synthesised by cells of the patient, into the patient.
- 6
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- 11
- 12 2. Use of a compound comprising an amino acid sequence of a protein for the treatment of an autoimmune disease in a patient, wherein the amino acid sequence is not homologous with amino acid sequences synthesised by cells of the patient.
- 13
- 14
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- 17
- 18 3. A composition for treatment of an autoimmune disease in a patient, comprising a compound which comprises an amino acid sequence of a protein which is not homologous with amino acid sequences synthesised by cells of the patient, in combination with a pharmaceutical carrier.
- 19
- 20
- 21
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- 24
- 25 4. The use of a compound comprising an amino acid sequence of a protein which is not homologous with amino acid sequences synthesised by the cells of a patient for the manufacture of a medicament for the treatment of an autoimmune disease in the patient.
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Patents Act 1977
Examiner's report to the Comptroller under
Section 17 (The Search Report)

Application number

9026278.3

Relevant Technical fields

Search Examiner

CI (Edition K) A5B (BHA)

C SHERRINGTON

CI (Edition 5) A61K 39/00, 37/02

Date of Search

3 FEBRUARY 1992

Databases (see over)

UK Patent Office

ONLINE DATABASES:WPI, DIALOG/PHARM

Documents considered relevant following a search in respect of claims

| Category see over) | Identity of document and relevant passages | Relevant to claim(s) |
|-----------------------|--|-------------------------|
| X | GB 2221157 A (BIOGAL GYOGYSZERGYAR) especially page 1, line 12-14; Claim 19 | 4 |
| X | EP 0322990 A1 (DE STAAT DER NEDERLANDEN..) whole document | 4 |
| X | WO 88/10120 A1 (BRIGHAM AND WOMEN'S HOSPITAL) whole document especially page 6, line 19 - page 7, line 3; Example 6; Claims 1-10, 13-19 | 4 |
| A | WO 85/05034 A1 (UNIVERSITY OF LONDON ET AL) especially page 2, line 18 - page 3, line 4; Claims 3-5 | 4 |
| X | Clin.exp.Immunal.1990,81,189-194 Prevention of adjuvant arthritis in rats by a nonapeptide from the 65-kd... | 4 |
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| X | Immunology 1969, 16 (2), 157-165 Inhibition of Adjuvant Arthritis by Protein Antigens | 4 |

| Category | Identity of document and relevant passages | Relevant to claim(s) |
|----------|--|----------------------|
| | | |

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